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2

3

8.

from both said first color and said second color.

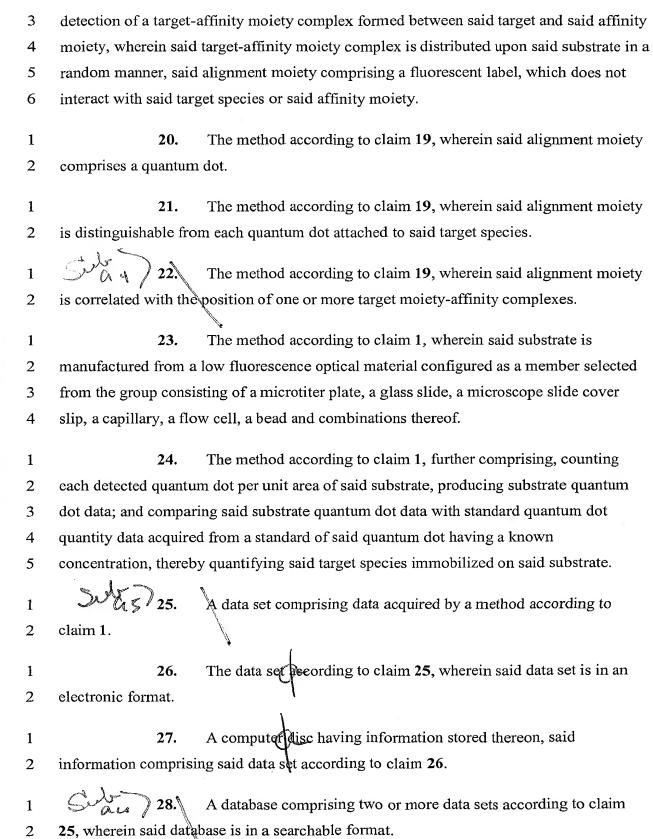
## WHAT IS CLAIMED IS:

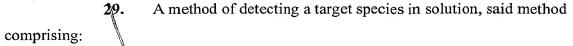
		WAR TO CEIMINADE IS.
	1	A method of detecting a target species immobilized on a substrate,
	2	said method comprising:
	3	detecting a single copy of said target species by detecting fluorescence
	4	emitted by a quantum dot attached to said single copy, wherein said single copy is bound
	5	to an affinity moiety for said target species immobilized on said substrate.
Sot	BI	2. The method according to claim 1, wherein said quantum dot is
	2	attached to said target species prior to binding said target species to said affinity moiety.
	1	3. The method according to claim 1, wherein said quantum dot is
	2	attached to said target species after binding said target species to said affinity moiety.
Sanctive Control of the Control of t	1	The method according to claim 1, wherein said target species has a
t.j	2	second quantum dot attached thereto and said first quantum dot is distinguishable from
The state of the s	3	said second quantum dot.
	] db {	5. The method according to claim 4, wherein binding of said target
·	2	species to said affinity moiety forms a target species-affinity moiety complex that is
	3	detected by fluorescence from both said first quantum dot and said second quantum dot
	4	attached to said target species-affinity moiety complex.
in in	1	6. The method according to claim 4, wherein said first quantum dot
	2	and said second quantum dot are distinguishable by a characteristic which is a member
	3	selected from the group consisting of fluorescence spectrum, fluorescence emission,
	4	fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance,
	5	fluorescence quantum yield, fluorescence lifetime, light scattering and combinations
	6	thereof.
	1	7. The method according to claim 4, wherein said first quantum dot
	2	and said second quantum dot are visually distinguishable as a first color and a second
	3	color, respectively.

second color combine to form a visually or electronically distinguishable color different

The method according to claim 7, wherein said first color and said

1	9. The method according to claim 1, wherein said target species has n
2	quantum dots attached thereto, wherein each of said $n$ quantum dots is distinguishable
3	from each other, and $n$ is an integer from 3 to 10.
1	The method according to claim 1, wherein said first quantum dot is
2	attached to a targeting moiety for said target species, said targeting moiety being a
3	member selected from the group consisting of antibodies,, aptamers, proteins,
4	streptavidin, nucleic acids and biotin.
1	11. The method according to claim 1, wherein said affinity moiety is
2	labeled with a quantum dot.
1	12. The method according to claim 1, wherein said target species is a
2	member selected from the group consisting of organisms, biomolecules and bioactive
3	molecules.
1	13. The method according to claim 1, wherein said affinity moiety is a
2	member selected from the group consisting of organisms, biomolecules and bioactive
3	molecules.
1	14. The method according to claim 1, wherein said substrate has bound
2	thereto a second affinity moiety.
1	15. The method according to claim 14, wherein said first affinity
2	moiety and said second affinity moiety are different affinity moieties.
1	16. The method according to claim 1, wherein said substrate has bound
2	thereto $m$ affinity moieties; and $m$ is an integer from 1 to 10,000.
1	17. The method according to claim 16, wherein each of said m affinity
2	moieties is a different affinity moiety.
1	18. The method according to claim $16$ , wherein said $m$ affinity
2	moieties are ordered in an array format.
1	19. The method according to claim 1, wherein said substrate further
2	comprises an alignment moiety providing a reference point on said substrate for the





detecting a single copy of said target species by detecting essentially simultaneously fluorescence emitted by a first quantum dot of a first color attached to said single copy and a second quantum dot of a second color attached to said single copy, wherein said first color and said second color are distinguishably different colors.

30. A method of detecting a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate with spacing between each member of said population, said method comprising:

detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, wherein said detecting is performed with a detecting means having a resolution that is higher than said spacing between each member of said population.

31. A method of detecting a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate forming a target-affinity moiety complex, and said detecting is performed with a detecting means having a resolution limited region of interest such that, in general, less than one target-affinity moiety complex is present within each resolution limited region of interest.

- 32. A method of detecting a first target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:
  - (a) defining a first region of interest of said substrate;
  - (b) probing said first region of interest for fluorescence emitted by a quantum dot attached to a single copy of said first target species bound to an affinity moiety for said first target species immobilized on said substrate, wherein said probing resolves said fluorescence

9		from said first target species from fluorescence arising from other
10		members of said population of target species immobilized on said
11		substrate.
1	33.	The most had according to plain 22 forther conversions data store a
1		The method according to claim 32, further comprising detecting a
2		s immobilized to said substrate, said method comprising:
3	. ,	efining a second region of interest of said substrate; and
4	(d) pr	obing said second region of interest for fluorescence emitted by a
5		quantum dot attached to said a single copy of said second target
6		species bound to an affinity moiety for said second target species
7		immobilized on said substrate, wherein said probing resolves said
8		fluorescence from said second target species from fluorescence
9		arising from other members of said population of target species
10		immobilized on said substrate.
1	34.	The method according to claim 33, wherein said first region of
2	interest and said seco	ond region of interest are the same region of interest.
1	35.	The method according to claim 32, wherein said probing is by a
2	method selected from	n the group consisting of microscopy, confocal fluorescence
3	microscopy and two-	-dimensional imaging with a CCD camera.
1	36.	The method according to claim 32, wherein said first target species
2	and said second targe	et species are different species.
1	Sul 37.	A method for detecting multiple target species immobilized on a
2		cies are members of a population of target species immobilized on
3	said substrate, said m	ethod comprising:
4	(a) de	fining multiple regions of interest on said substrate; and
5	(b) pr	obing said multiple regions of interest for fluorescence emitted by a
6		quantum dot attached to a single copy of said target species bound
7		to an affinity moiety for said target species immobilized within a
8		region of interest of said substrate, wherein said probing resolves
9		fluorescence from said multiple target species from other members
10		of said population and from each other.

1	38. A method for determining whether a target species within a region
2	of interest on a substrate is quantifiable by a technique selected from the group consisting
3	of single target counting and ensemble counting, said method comprising:
4	(a) probing said region of interest to determine target species density
5	within said region of interest by detecting fluorescence emitted by
6	a quantum dot attached to one or more molecules of said target
7	species bound to an affinity moiety for said target species
8	immobilized on said substrate;
9	(b) comparing said density to a predetermined density cutoff value above
10	which ensemble counting is used and below which single target
11	counting is used.
1	39. The method according to claim 38, wherein said substrate
2	comprises a first region in which ensemble counting is used and a second region in which
3	single target counting is used.

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